

REMARKS

Upon entry of the amendments, claims 4-6 and 30-35 are pending. Claims 4 and 5 are amended. Support can be found at page 21, line 19 - page 23, line 13. No new matter is added.

Rejection under 35 U.S.C. §112, First Paragraph

Claims 4-6 and 30-35 are rejected under 35 U.S.C. §112, first paragraph for failing to comply with the enablement requirement. The Examiner states that Applicants have not fully enabled the claimed invention for the full scope of the claim. Specifically, the Examiner states that the claims are drawn to “conditions” that allow the formation of S-nitrosohemoglobin (SNOHb). However, the Examiner asserts that specific conditions are necessary to make the desired end product and the instant specification does not describe all the desired conditions to achieve SNOHb. *See*, Office Action at pages 2-3. As such, the Examiner contends that the one of ordinary skill in the art would be burdened with undue experimentation to determine the appropriate conditions for obtaining SNOHb rather than obtaining methemoglobin (metHb) or iron-nitrosylhemoglobin.

Claims 4 and 5 are amended to recite “under conditions sufficient to preserve redox chemistry in hemoglobin.” Applicants traverse the rejection with respect to the claims as amended herein.

The present invention is directed to the novel discovery that nitric oxide (NO) binding to oxygenated hemoglobin (oxyHb) is cooperative *in vivo*, as previous studies have suggested such interaction is non-cooperative. *See*, specification at page 15, line 22 - page 16, line 12. The present invention describes that the distribution of the hemoglobin population having vacancies on the hemes controls the function of hemoglobin. Thus, the present invention provides that by regulating the functional behavior of this vacancy population, hemoglobin can either a) quench and eliminate excess nitric oxide (*i.e.*, metHb), b) store excess nitric oxide in a form that is not a donor of NO (*i.e.*, iron nitrosylhemoglobin), or c) store NO in a form that donates NO (*i.e.*, SNOHb).

Specifically, as described at page 22, lines 8-19, the invention provides methods for quenching of NO (that is, consumption of NO by Hb and oxidizing NO to nitrate with

formation of metHb) by minimizing vacancies of molecules in R structure (*e.g.*, R3) whilst maximizing oxyhememes of molecules in T structure (*e.g.*, T1). The invention provides also provides methods for storage of NO in non-donor form, as iron nitrosylhemoglobin, by maximizing vacancies of molecules in R structure (*e.g.*, maximize R3 state) while minimizing oxyligated hemes on hemoglobins in T state (*e.g.* T1). Most importantly, the present invention provides methods for storage in bioactive form as donors of NO, as SNOHb, by maximizing vacancies of molecules in R structure (*e.g.*, maximize R3 state) while minimizing oxyligated hemes on hemoglobins in T state (*e.g.* T1) and requiring that the vacancies can undergo redox chemistry, without which NO cannot transfer from heme to thiol. Redox chemistry is well known in the art and refers to the transfer of NO from the heme Fe to cysteine on the β subunit with the loss of an electron.

Thus as described at page 22, line 20 - page 23, line 5, in a simple competition model, cooperativity of NO binding identifies the storage of NO in non-donor form, as iron nitrosylhemoglobin, whereas lack of cooperativity identifies the quenching of NO with the formation of metHb. In addition, it is demonstrated that cooperativity of NO binding is not sufficient for transformation of NO into bioactive form; but rather, by regulating the auto-oxidation function of hemoglobin in vacancies or by adding redox modifiers such as nitrite, one can greatly enhance the transformation into SNOHb. Thus as the examples describe, high phosphate conditions result in the predominate formation of metHb, low phosphate conditions result in the predominate formation of iron nitrosylhemoglobin and low phosphate conditions which preserve redox chemistry in hemoglobin result in the predominate formation of SNOHb. *See*, specification at page 22, line 20 - page 23, line 13.

For the foregoing reasons, Applicants submit that one of ordinary skill in the art, provided with the teaching of the instant specification, would readily determine the appropriate conditions for obtaining SNOHb without the need for undue experimentation. Applicants respectfully request the rejection be withdrawn.

CONCLUSION

On the basis of the foregoing amendment and remark, Applicants respectfully submit that the pending claims are in condition for allowance. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Matthew Pavao", written over a horizontal line.

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Dated: March 30, 2006